

WHAT IS CLAIMED IS:

1. A method for determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:
 - obtaining a sample comprising said target sequence;
 - 5 hybridizing a primer upstream of said polymorphic nucleotide;
 - performing a first extension reaction with said hybridized primer in the absence of a deoxyribonucleoside triphosphate or ribonucleoside triphosphate complementary to said first known variant;
 - performing a second extension reaction with said hybridized primer in the absence of a deoxyribonucleoside triphosphate or ribonucleoside triphosphate complementary to said second known variant; and
 - 10 determining the length of said primer following said first extension reaction and said second extension reaction.
2. The method of claim 1 wherein a plurality of dNTPs or rNTPs is included in said first and second extension reactions.
3. The method of claim 1 wherein only one dNTP or rNTP is included in said first and second extension reactions.
4. The method of claim 1 wherein said primer hybridizes such that its 3' end is immediately upstream of the polymorphic base.
- 20 5. The method of claim 4 wherein one dNTP or rNTP is added.
6. The method of claim 1 wherein said primer is labeled at its 5' end.
7. The method of claim 1, wherein said target sequence is amplified *in vitro*.
8. The method of claim 1, wherein the reaction products are detected using high performance liquid chromatography.
- 25 9. The method of claim 1, wherein the reaction products are detected using capillary electrophoresis.
10. The method of claim 1, wherein the reaction products are detected using an intercalating agent.
- 30 11. The method of claim 10, wherein said intercalating agent is ethidium bromide.

12. The method of claim 10, wherein intercalating agent is an unsymmetrical cyanine dye.

13. The method of claim 1, wherein the reaction products are detected using slab electrophoresis and ultraviolet light.

5 14. The method of claim 1, wherein the reaction products are detected using slab electrophoresis and a DNA-binding dye.

15. The method of claim 1 wherein the biallelic markers being analyzed are associated with genetic disorders.

10 16. The method of claim 1, wherein said sample containing a target sequence having at least two known variants is from a diploid organism.

15 17. The method of claim 1, wherein said first extension reaction is performed with a primer having a first length, and said second reaction is performed with a primer having a second length, said first and second lengths being selected such that said first primer and said second primer and any extension products thereof, can be distinguished from one another.

18. The method of claim 17, wherein the reaction products of said first and second extension reactions are analyzed separately.

19. The method of claim 17, wherein the reaction products of said first and second extension reactions are pooled for analysis.

20 20. A method for screening a DNA sample for a plurality of target sequences having at least two known variants, comprising:

25 obtaining a sample comprising a plurality of known target sequences;
hybridizing a primer upstream of each of said target sequences, each primer having a length such that said primer and any extension product thereof can be distinguished from the other primers and any extension products thereof;

performing a plurality of extension reactions wherein each extension reaction contains a single free dNTP or rNTP species complementary to one polymorphic nucleotide of said variant; and

30 determining the lengths of each of said primers following each extension reaction.

21. The method of claim 20 wherein said target sequences being analyzed are associated with genetic disorders.

22. The method of claim 20, wherein said sample is from a diploid organism.

23. The method of claim 20, wherein the products of the extension reactions are analyzed separately.

24. The method of claim 20, wherein the products of the extension reactions are pooled for analysis.

25. A kit for use in determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:

at least one primer that hybridizes to said target sequence such that its 3' end is upstream of said target sequence;

a reagent for performing a primer extension reaction in the absence of a dNTP or rNTP complementary to said first known polymorphic nucleotide; and

a reagent for performing a primer extension reaction in the absence of a dNTP or rNTP complementary to said second known polymorphic nucleotide.

26. The kit of claim 25 further comprising a detection enhancer for said reaction products.

27. The kit of claim 25 further comprising a purifier for reaction products.

28. The kit of claim 27 further comprising a detection enhancer for said reaction products.

29. A kit for use in determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising:

at least one primer that hybridizes to said target sequence such that its 3' end is immediately upstream of said target sequence;

a reagent for performing a primer extension reaction containing a single dNTP or rNTP complementary to said first known polymorphic nucleotide; and

a reagent for performing a primer extension reaction containing a single dNTP or rNTP complementary to said second known polymorphic nucleotide.

30. The kit of claim 29 further comprising a detection enhancer for said reaction products.

31. The kit of claim 29 further comprising a purifier for reaction products.

32. the kit of claim 31 further comprising a detection enhancer for said reaction products.

5 33. A method for determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising performing a primer extension reaction in the absence of a dNTP or rNTP complementary to one of said polymorphic nucleotides and determining the length of said primer following said extension reaction.

Add As

0947103-12399